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From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 04 August 1997 (04.08.97)	
International application No. PCT/NO96/00266	**Applicant's or agent's file reference** Eij 1 HV
International filing date (day/month/year) 13 November 1996 (13.11.96)	**Priority date** (day/month/year) 13 November 1995 (13.11.95)
Applicant EIJSINK, Vincent, G., H. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

03 June 1997 (03.06.97)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

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Authorized officer

F. Gateau

Telephone No.: (41-22) 338.83.38

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 96/00266

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 15/74, C07K 14/335
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, FULLTEXT, MEDLINE, BIOSIS, DERWENT BIOTECH ABS, EMBL/GENBANK/ SWISSPROT/DDBJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Molecular Microbiology, Volume 18, No 4, 1995, Dzung Bao Diep et al, "A bacteriocin-like peptide induces bacteriocin synthesis in Lactobacillus plantarum C11" page 631 - page 639 --	1-15,17-21
X	Microbiology, Volume 140, 1994, Petra S. Tichaczek et al, "Cloning and sequencing of sakP encoding sakacin P, the bacteriocin produced by Lactobacillus sake LTH 673", page 361 - page 367, see especially fig 2 --	1-15,17-21

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 1997	28 -02- 1997
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 96/00266

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Molecular Microbilogy, Volume 17, No 3, 1995, Koen Venema et al, "Functional analysis of the pediocin operon of Pediococcus acidilactici PAC1.0: PedB is the immunity protein and PedD is the precursor processing enzyme", page 515 - page 522, fig 1 and summary	1-15,17-21
X	Applied and EnvironmentalMicrobiology, Volume 60, No 1, January 1994, Dzung Bao Diep et al, "The Gene Encoding Plantaricin A, a Bacteriocin from Lactobacillus plantarum C11, Is Located on the Same Transcription Unit as an agr-Like Regulatory System" page 160 - page 166	1-15,17-21
X	Journal of Bacteriology, Volume 177, No 8, April 1995, Lars Axelsson et al, "The Genes Involved in Production of and Immunity to Sakacin A, a Bacteriocin from Lactobacillus sake Lb706" page 2125 - page 2137	1-2,6-15, 17-21
X	Applied and Environmental Microbiology, Volume 57, No 2, February 1991, Marco J. van Belkum et al, "Organization and Nucleotide Sequences of Two Lactococcal Bacteriocin Operons", page 492 - page 498, figure 1	1-2,6-15, 17-21
X	EP 0493779 A1 (QUEST INTERNATIONAL B.V.), 8 July 1992 (08.07.92), page 3, line 24 - line 31	1-2,6-15, 17-21
X	WO 9404682 A1 (DZIEGLEWSKA, HANNA, EVA), 3 March 1994 (03.03.94), page 11, line 12 - line 37	1-2,6-15, 17-21
X	WO 119802 A1 (HOLMES, MICHAEL, J.), 25 December 1991 (25.12.91), page 6, line 9 - line 36	1-2,6-15, 17-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter...tional application No.
PCT/NO 96/00266

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0493779	08/07/92	AU-B- 638616	01/07/93
		AU-A- 8820091	09/07/92
		CA-A- 2056086	01/07/92
		JP-A- 7067652	14/03/95
		US-A- 5175252	29/12/92
		US-A- 5260212	09/11/93
WO-A1- 9404682	03/03/94	AU-A- 4968893	15/03/94
WO-A1- 119802	25/12/91	NONE	

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
Eij 1 im		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NO96/00266	13/11/1996	13/11/1996
International Patent Classification (IPC) or national classification and IPC		
C12N15/74		
Applicant		
Eijsink, Vincent G.H. et Al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/06/1997	24.02.98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Huber, A Telephone No. (+49-89) 2399-8173

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NO96/00266

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-27 as originally filed

Claims, No.:

1-15 with telefax of 16/02/1998

Drawings, sheets:

1/3-3/3 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-15
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

SECTION V ----------

1. The present application relates to an inducible gene expression system in lactic acid bacteria. It has been discovered that expression of the IF-K-R gene cluster is autoinduced by the gene product of the IF gene.

2. The quoted documents are:

   D1: P. Tichaczek et al., Microbiology, Vol. 140, pp. 361-367, 1994

   D2: Axelsson et al., Journal of Bacteriology, Vol. 177, No. 8, pp. 2125-2137, 1995

3. D1 discloses the sequence of bacteriocin SakP, its promoter and two further open reading frames (orfX and orfY), whose function is unknown. It is speculated that these ORFs could encode immunity proteins.

   D2 is concerned with an operon containing the genes necessary for production and immunity of the bacteriocin sakacin A (SapA) from *Lactobacillus sake* Lb706. It is assumed that proteins SapK and SapR constitute a two-component signal-transduction system, mediating a response to an environmental signal, while SapT and SapE seem to be involved in secretion of SapA. SaiA encodes the immunity peptide. Two further open reading frames were identified (Orf4 and Orf1) but no function has been accorded to them. Both are similar to the bacteriocin leader peptides and are speculated to be structural genes for bacteriocins.

4. Claim 1 relates to a gene expression system in lactic acid bacteria which is characterised in that it comprises a gene/genes of interest which have been operably linked by genetic engineering to a strongly regulated promoter whose activity can be induced by an unmodified peptide. The promoter elements and the

peptide are defined in the claim by all essential elements thereof. It is also specified in the claim that the genes of interest are **not identical** to the genes that are operably linked to said promoter elements in the lactic acid bacteria from which said promoter elements are derived.
The latter feature distinguishes the claimed gene expression system from naturally occurring lactic acid bacteria which inherently comprise the promoter elements specified in the claim.

A gene expression system as defined in Claim 1 has neither been disclosed nor rendered obvious in any of the cited documents. The subject-matter of Claim 1 and of the claims which are directly or indirectly dependent thereon is therefore considered novel and also appears to involve the required inventive step (Article 33(2) and (3) EPC).

5. Claim 11 relates to a peptide which is defined by its amino acid sequence. The peptide is capable of activating the inducible promoter described in Claim 1. Said peptide has not been disclosed in the prior art and its sequence could not have been derived plainly and logically from any of the cited documents. Novelty and inventive step are therefore recognized for the subject-matter of Claim 11 (Article 33(2) and (3) PCT).

CLAIMS

1. Gene expression system,
characterized in that it comprises genes, promoter sequences and peptides involved in the production of bacteriocins except nisin in lactic acid bacteria.

2. Gene expression system,
characterized in that it contains at least one specific regulated promoter, genes involved in transducing signals that induce gene expression, a peptide being that signal, and, possibly, genes involved in producing, processing, and secreting this inducing peptide.

3. The expression system of claims 1 and 2,
characterized in that the said peptide is capable of inducing its own production and/or that of one or more bacteriocins by the lactid acid bacteria.

4. The expression system of claim 3,
characterized in that said peptide is a functional analogue of the peptide of claim 3, functional analogues being defined as shortened, enlarged or mutated variants that retain the potential to induce gene expression.

5. The expression system of claim 4,
wherein said peptide has the sequence Met-Ala-Gly-Asn-Ser-Ser-Asn-Phe-Ile-His-Lys-Ile-Lys-Gln-Ile-Phe-Thr-His-Arg, (seq. id. no. 1) or has the sequence Lys-Ser-Ser-Ala-Tyr-Ser-Leu-Gln-Met-Gly-Ala-Thr-Ala-Ile-Lys-Gln-Val-Lys-Lys-Leu-Phe-Lys-Lys-Trp-Gly-Trp (seq. id. no. 2).

6. The expression system of claim 1,
characterized in that said promoter has a DNA sequence that is essentially similar to the promoter elements shown in Figure 4, essentially similar being defined by the presence of direct repeats, TATA boxes and a characteristic spacing between these elements and by the fact that expression initiated at this promoter can be induced by a mechanism similar to the mechanism for the induction of expression of the genes shown in Figure 1.

7. The expression system of claim 6, wherein said promoter has the DNA sequence of one of the promoter elements shown in Figure 4.

Replaced by Article 34

8. The expression system of claims 1-7,
characterized in that one or more of the said genes are selected from the group of the genes denoted IF, K, R, P, I, T, A in Fig. 1, or the gene/genes are analogous of these genes, analogous genes being defined as genes that bear sequence homology with IF or K or R or P or I or T or A, are isolated from lactic acid bacteria, and are involved in bacteriocin production and/or the regulation thereof in those bacteria, and the said peptide is one of the peptides described in claims 3-5, and that the said promoter is one of the promoters described in claims 6 and 7.

9. The expression system of claim 1,
characterized in that the said genes are at least one or more genes selected from the group the genes denoted IF, K, R, T, A, P, and I in Fig. 1, the said promoter has the DNA sequence of one of the promoters according to claim 6 and 7 and the said peptide is Met-Ala-Gly-Asn-Ser-Ser-Asn-Phe-Ile-His-Lys-Ile-Lys-Gln-Ile-Phe-Thr-His-Arg (seq. id. no. 1), or any combination of two or more of the above genes.

10. A recombinant vector,
characterized in comprising any possible combination of genes and promoter elements that are part of claim 1-9, preferably in that it contains a promoter element with the DNA sequence of one of the promoter elements described in claims 6 and 7 operably linked to a gene encoding a desired protein of interest.

11. A host cell,
characterized in that it is transformed with the recombinant vector of claim 10 and contains any possible combination of genes and promoter elements that are part of claims 1-9 integrated in the chromosome, and/or in that also integrated in its chromosome is a promoter element with the DNA sequence of one of the promoter elements described in claims 3 and 4 operably linked to an also integrated gene encoding a desired protein of interest.

12. The host cells of claim 11,
characterized in that some of the said genes and promoter elements are present in plasmids and some are present in the chromosome.

13. The host cells of claims 11-12,

characterized in that the host is a Gram-positive bacterium, preferably a lactic acid bacterium.

14. The host cell of claims 11-13,
characterized in that said host possesses the food consumption classification of GRAS (Generally Regarded As Safe).

15. The host cells of claims 11-14,
characterized in that it is selected from the group consisting of members of the genera *Lactobacillus, Lactococcus, Pediococcus*, preferably members of the genus Lactobacillus, more preferably of *Lactobacillus sake* and *Lactobacillus plantarum*, most preferably of *Lactobacillus sake* LTH673 and *Lactobacillus plantarum* C11.

16. Purified peptide,
characterized in that it has the amino acid sequence of Met-Ala-Gly-Asn-Ser-Ser-Asn-Phe-Ile-His-Lys-Ile-Lys-Gln-Ile-Phe-Thr-His-Arg (seq. id. no. 1).

17. Purified protein,
characterized in that it is produced by any of the host cells of claims 11-15 after or not addition of any of the peptides of claims 3-5.

18. Use the gene expression system according to claim 1-9, in any of the host cells described in claims 11-15 to induce gene expression by adding any of the peptides described in claims 3-5.

19. Use of any of the host cells of claims 11-15 in fermentations.

20. Use of any of the host cells of claims 11-15 to produce a desired protein of interest.

21. A kit for using the expression system according to claim 1, in lactid acid bacteria, **characterized in** consisting of:

1) One or more recombinant vectors each vector containing a promotor element identical or similar to one of the promoter elements depicted in Fig. 4, directly followed by a multiple cloning site; these vectors may also contain one or more genes selected from the group K, R, IF, T, A (Fig. 1) or functional analogues of these genes,

2) Lactic acid bacteria that can function as host strain for these recombinant vectors, and that, depending on the recombinant vector used, may contain one or more genes selected from the group K, R, IF, T, A (Fig. 1) (or functional analogues of these genes) integrated in the chromosome,

3) A peptide that is capable of inducing the expression of genes under control of promoter elements similar or identical to the promoter elements depicted in Fig. 4 and that needs a two component system similar or identical to that encoded by genes K and R (Fig. 1) to exert its inducing action.

[redacted]

CLAIMS

1. Gene expression system,
characterized in that it comprises a gene/genes of interest that by genetic engineering have been operably linked to a strongly regulated promoter whose activity can be induced by an unmodified peptide, wherein said promoter and peptide are functional equivalent to promoters and peptides involved in the production of bacteriocins, except nisin, in lactic acid bacteria, and in that the products of two regulatory genes encoding a so called two-component regulatory system are essential for transducing the signal provided by said peptide into a change in activity of said strongly regulated promoter, and in that in naturally occuring lactic acid bacteria said regulatory genes are co-transcribed or closely associated with genes encoding said peptide, wherein the said peptide is a functional analogue of the peptide having the sequences shown in Seq. id. No. 1 and Seq. id. No. 2, and in that said gene/genes of interest are not identical to the genes that are operably linked to said promoter elements in the lactic acid bacterium from which said promoter elements are derived.

2. Gene expression system according to claim 1,
characterized in that said peptide is capable of inducing its own production and/or the production of one or more bacteriocins in lactic acid bacteria.

3. Gene expression system according to claims 1-2,
characterized in that said peptide is identical to the peptide having the sequences of Seq. id. No. 1 and Seq. id. No. 2.

4. Gene expression system according to claims 1-3,
characterized in that said promoter is identical or functionally analogous to the promoter elements shown in Fig. 4.

5. A recombinant vector,
characterized in that it comprises the gene/genes operably linked to the promoter elements according to claim 1-4, wherein this gene/these genes are not identical to the genes that are operably linked to said promoter elements in the lactic acid bacterium from which said promoter elements are derived.

6. A host cell,
characterized in that it contains the gene/genes of claim 5 operably linked to the promoter, and in that the expression of the said gene/genes can be regulated by adding a peptide according to claims 1-4.

7. The host cells of claim 6,
characterized in that some of the said genes and promoter elements are present in plasmids and some are present in the chromosome.

8. The host cells of claims 6-7,
characterized in that the host is a Gram-positive bacterium, preferably a lactic acid bacterium.

9. The host cell of claims 6-8,
characterized in that said host possesses the food consumption classification of GRAS (Generally Regarded As Safe).

10. The host cells of claims 6-9,
characterized in that it is selected from the group consisting of members of the genera *Lactobacillus, Lactococcus, Pediococcus*, preferably members of the genus Lactobacillus, more preferably of *Lactobacillus sake* and *Lactobacillus plantarum*, most preferably of *Lactobacillus sake* LTH673 and *Lactobacillus plantarum* C11.

11. Peptide,
characterized in that it has the amino acid sequence of Met-Ala-Gly-Asn-Ser-Ser-Asn-Phe-Ile-His-Lys-Ile-Lys-Gln-Ile-Phe-Thr-His-Arg (seq. id. no. 1).

12. Use the gene expression system according to claim 1-4, in any of the host cells described in claims 6-10 to induce gene expression by adding any of the peptides described in claim 3.

13. Use of any of the host cells of claims 6-10 in fermentations.

14. Use of any of the host cells of claims 6-10 to produce a desired protein of interest.

AMENDED SHEET

15. A kit for using the expression system according to claim 1, in lactid acid bacteria, characterized in consisting of:

1) One or more recombinant vectors each vector containing a promotor element identical or similar to one of the promoter elements depicted in Fig. 4, directly followed by a multiple cloning site; these vectors may also contain one or more genes selected from the group K, R, IF, T, A (Fig. 1) or functional analogues of these genes,

2) Lactic acid bacteria that can function as host strain for these recombinant vectors, and that, depending on the recombinant vector used, may contain one or more genes selected from the group K, R, IF, T, A (Fig. 1) (or functional analogues of these genes) integrated in the chromosome, such that at least the genes K and R or functional analogues thereof are present in said lactic acid bacteria containing said recombinant vector.

3) A peptide that is capable of inducing the expression of genes under control of promoter elements similar or identical to the promoter elements depicted in Fig. 4 and that needs a two component system similar or identical to that encoded by genes K and R (Fig. 1) to exert its inducing action.